

Review Article

# Mesenchymal Stromal Cells for Graft-Versus-Host Disease : Basic Aspects and Clinical Outcomes

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Mesenchymal stromal cells (MSCs) have unique characteristics such as immune suppression by inhibiting T cell proliferation, tissue-repair ability and acceleration of hemopoietic stem cell engraftment. The cells are rare in bone marrow, but easily cultured under standard culture conditions. Soluble factors and cells are implicated in the MSC-mediated T cell suppression and numerous clinical trials using MSCs to prevent and treat graft-versus-host disease (GVHD) have been reported. MSCs are suggested to suppress acute GVHD without impairing graft-versus-leukemia effects and increasing systemic infections. In this review, we focus on basic aspects of MSC-mediated T cell suppression and clinical trials using MSCs for GVHD and related conditions. [*J Clin Exp Hematopathol* 50(2) : 79-89, 2010]

**Keywords:** mesenchymal stromal cells, bone marrow, immunosuppression, graft-versus-host disease

## INTRODUCTION

Mesenchymal stromal cells (MSCs) are non-hemopoietic cells with the capacity to self-renew and differentiate into various cell lineages of mesenchymal origin.<sup>1</sup> These cells can be obtained from bone marrow, adipose tissues, fetal liver, and umbilical cord blood.<sup>2-4</sup> MSCs have great expansive potential under optimal conditions *in vitro*. After a 2-3 day incubation of human bone marrow aspirate, colonies of plastic-adherent spindle-shaped cells can be found (Fig. 1). Functionally, adult MSCs are characterized by rapid proliferation (a doubling time of 33 hr).<sup>5</sup> Although it has been estimated that MSCs constitute only 0.01%-0.001% of bone marrow cells, as many as 50-375 million MSCs can be generated by the passages from a 10-mL human bone marrow aspirate, and still retain their capacity for differentiation.<sup>1</sup> MSCs are expected to be a source of regenerative medicine for repairing defects in a variety of diseases. In children with osteogenesis imperfecta, allogeneic bone marrow-derived MSCs engrafted and stimulated growth.<sup>6</sup> Also, MSCs play a key role in the maintenance of the bone marrow microenvironment and regulate the maturation of hemopoietic stem cells by providing various growth factors. Promotion of engraftment and hema-

tological recovery after the co-infusion of autologous hematological stem cells and MSCs were reported.<sup>7-9</sup>

More recently, the immune regulatory potential of MSCs has been focused on. MSCs have been found to suppress inflammation by inhibiting T cell proliferation, representing a novel treatment for graft-versus-host disease (GVHD). Le Blanc *et al.* described a patient with severe refractory stage IV GVHD of the gut and liver who was infused with MSCs in 2004.<sup>10</sup> His GVHD improved dramatically and rapidly following 2 infusions, and no significant side effects occurred. In a multicenter phase II study by the European Group for Blood and Marrow Transplantation, the response rate to treatment of GVHD with MSCs was over 70%, and treatment efficiency was not related to a donor human leukocyte antigen (HLA)-match.<sup>11</sup> However, the molecular mechanisms by which MSCs suppress immune responses *in vivo* and *in vitro* are poorly understood. We here review the molecular mechanisms of immunomodulation by MSCs and results of clinical trials using the cells.

## BASIC ASPECTS

### *Immune regulation by MSCs*

First, it should be emphasized that there are distinct differences in immune suppressive activity between human and non-human derived MSCs.<sup>12</sup> Regardless of species though, MSCs exert strong immune suppressive activity against a broad range of immune cells. However, the rate of cell growth, cell surface antigens, and soluble factors implicated in MSC-mediated immune suppression vary (data not

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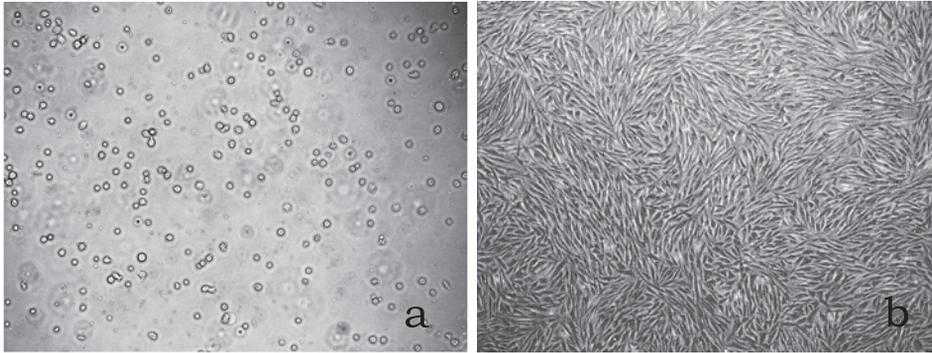
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**Fig. 1.** Bone marrow-derived mesenchymal stromal cells on phase contrast microscope. After incubation of human bone marrow aspirate for 2 days, adherent cells appear (*1a*) and they rapidly grow at 14 days (*1b*). (*1a*) & (*1b*)  $\times 40$ .

shown).<sup>12,13</sup> Despite the great interest in MSCs, a clear definition of MSCs has not been established, and plastic-adherent cells from bone marrow cultures are highly heterogeneous. Human MSCs can be relatively easily isolated and rapidly expanded. In contrast, murine MSCs are difficult to propagate and usually contaminated by hemopoietic precursors (data not shown).<sup>14</sup> Furthermore, methods of isolation and expansion differ among investigators. Therefore, results regarding the immune suppressive mechanisms of MSCs should be interpreted carefully. MSCs have been shown to inhibit not only T cells<sup>15-20</sup> but also B cells,<sup>18,20,21</sup> natural killer cells,<sup>22</sup> and monocyte-derived dendritic cells.<sup>23</sup> As the T cell inhibition by MSCs has been investigated, we focus here on the molecular mechanism of this inhibition.

### Conventional T cells

The idea of investigating the immune suppressive effects of MSCs on T cell responses comes from the role of the thymic epithelium in T cell development.<sup>24</sup> Hemopoietic stem cells reside in bone marrow niches surrounded by MSCs which regulate the self-renewal and differentiation. However, little has been investigated about T cell regulation by MSCs. In the presence of MSCs, T cell responses stimulated by alloantigens (e.g., mixed lymphocytes),<sup>15,17,19</sup> peptide antigens,<sup>16,18</sup> mitogens,<sup>15,19,20</sup> and a CD3/CD28 antibody<sup>20</sup> have been tested, suggesting that the immune suppressing effects of MSCs are not antigen-specific. The inhibitory effects of MSCs on T cell proliferation are dose-dependent.

Phorbol 12-myristate 13-acetate and ionomycin are known to act downstream of the T cell receptor complex by activating protein kinase C and inducing  $\text{Ca}^{2+}$  influx, respectively. T cell proliferation stimulated by these mitogens was also suppressed by MSCs, suggesting that the T cell receptor complex is not a target for the suppression and that MSCs influence signals downstream of protein kinase C and  $\text{Ca}^{2+}$  influx.<sup>20</sup> As MSCs equally inhibit the proliferation of both CD4 and CD8-

positive T cells as well as unfractionated T cells, the inhibitory effects of MSCs do not target any specific T cell subpopulations.<sup>15,18,20</sup> The transwell system, by which one can physically separate T cells and MSCs with a finely textured permeable membrane, has been used to confirm the necessity of cell-contact. However, T cell-MS-C-contact dependency is still controversial.<sup>15-20</sup> Di Nicola *et al.* initially reported that T cell proliferation was also significantly inhibited using the transwell system, thus suggesting that a soluble factor is involved. However, the rate of T cell inhibition increased when contact between MSCs and T cells was allowed.<sup>15</sup> These results have been also confirmed in our laboratory.<sup>20</sup> Taken together, cell-contact could be required for maximum T cell suppression by MSCs, but soluble factors secreted by MSCs have recently been considered to play a key role in MSC-mediated immune suppression.

So far, transforming growth factor- $\beta 1$ , hepatocyte growth factor, indoleamine 2, 3-dioxygenase, which induces tryptophan's catabolism, prostaglandin E2 (PGE2), and nitric oxide (NO) have been reported to mediate the T cell inhibition by MSCs (Table 1).<sup>15,17,19,20</sup> Djouad *et al.* found that conditioning medium obtained from MSCs cocultured with "activated (stimulated)" splenocytes suppressed T cell proliferation, whereas the supernatant from neither cultures of MSCs alone nor MSCs cocultured with "resting (non-stimulated)" splenocytes inhibited T cell proliferation, suggesting that the "activation" of MSCs by T cells was required for T cell suppression.<sup>25</sup> Some reports have shown that MSCs suppressed the expression of the early activation markers CD25 (IL-2 receptor) and CD69,<sup>15,18,20</sup> whereas others have demonstrated that MSCs had little or no effect on the activation markers.<sup>26,27</sup> Division arrest anergy of activated T cells induced by MSCs was also reported. Glennie *et al.* have shown that the expression of cyclin D2 was prevented, whereas the expression of the negative cell cycle regulatory protein p27<sup>kip1</sup> was strongly downregulated in stimulated T cells co-cultured with MSCs.<sup>18</sup> Analysis of the cell cycle showed that T cells,

**Table 1.** Mesenchymal stromal cell (MSC)-mediated immune suppression

1st author	Origin of MSCs	Source of MSCs	Necessity of cell-contact	Immunosuppressive factor(s) or mechanism	Reference
Di Nicola	human	bone marrow	partially required	TGF- $\beta$ , HGF	15
Krampera	mouse	bone marrow	required	unmentioned	16
Meisel	human	bone marrow	not examined	IDO	17
Glennie	mouse	bone marrow	not examined	division arrest anergy	18
Aggarwal	human	bone marrow	not examined	PGE2	19
Sato	mouse	bone marrow	partially required	nitric oxide, PGE2	20

TGF- $\beta$ , transforming growth factor- $\beta$ ; HGF, hepatocyte growth factor; IDO, indoleamine 2, 3-dioxygenase, PGE2, prostaglandin E2

stimulated in the presence of MSCs, were arrested at the G1 phase.<sup>18</sup> These investigators argued that the inhibition of T cell proliferation was profound and irreversible.<sup>18</sup> However, Krampera *et al.* and we have shown that although the presence of MSCs inhibited the first antigenic stimulation, when MSCs were removed the response to the second stimulation was restored.<sup>16,20</sup> Recently, we have reported that the STAT5 phosphorylation in T cells was suppressed in the presence of MSCs and that NO is involved in the suppression of STAT5 phosphorylation and T cell proliferation.<sup>20</sup> However, MSCs from inducible NO synthase knockout mice could still suppress T cell proliferation. Furthermore, indomethacin (inhibitor of PGE2 production) also restored T cell proliferation, but the effects of a specific inhibitor of NO synthase and indomethacin were not additive. These findings suggest that the molecular mechanisms of T cell inhibition by MSCs involve various factors in response to inflammatory cytokines, and that the cell-signaling pathway is also complicated.

### *Th1/Th2 and Th17*

The importance of the T helper (Th)1/Th2 balance has been well established in GVHD. In some experimental models, Th1 cells augment and Th2 cells ameliorate acute GVHD.<sup>28,29</sup> A previous report by our colleagues confirmed that mouse MSCs suppressed both the proliferation and differentiation of Th1 cells, whereas the suppression of Th2 cells was mild.<sup>30</sup> Aggarwal *et al.* also showed that human MSCs caused Th1 cells to secrete less interferon- $\gamma$  and caused Th2 cells to increase secretion of interleukin (IL)-4.<sup>19</sup> These results suggested that MSCs interact with T cells and induce a Th1 to Th2 shift. Recently, we identified a novel T cell subset, namely, CD4 T cells which produce the proinflammatory cytokine IL-17. Regulatory T (Treg) cells positive for CD4 and CD25 are another newly recognized subset, in which the CD4 T cells have high levels of Foxp3 expression and inhibit T cell proliferation. Treg cells prevented GVHD by inhibiting the proliferation and function of conventional T

cells in a murine model,<sup>31</sup> whereas the role of Th17 cells in the pathogenesis of GVHD is still unknown.<sup>32,33</sup> Very recently, we showed that MSCs block the differentiation of Th17 cells through PGE2 production.<sup>34</sup>

## CLINICAL OUTCOMES

### *MSCs for steroid-resistant acute GVHD*

A summary of published reports on the treatment of steroid-resistant acute GVHD (aGVHD) with MSCs is shown in Table 2. The first case of severe aGVHD successfully treated with MSCs was reported by LeBlanc *et al.*<sup>10</sup> The patient, a 9-year-old boy with acute lymphoblastic leukemia (ALL) in his third remission, received a peripheral blood stem cell transplant from an HLA-identical unrelated female donor. After the transplantation, the patient developed grade IV aGVHD of the liver and gut, which did not respond to conventional doses of steroid, bolus steroid, infliximab, daclizumab, and mycophenolate mofetil or other treatments. MSCs were prepared from his haploidentical mother's bone marrow and infused twice into the patient. The patient's aGVHD completely disappeared. Importantly, in the authors' institution, this individual was the only surviving patient among 25 patients with grade IV aGVHD after hemopoietic stem cell transplantation (HSCT). Ringden *et al.* that reported eight adults received MSCs for steroid-resistant aGVHD.<sup>35</sup> The MSCs were prepared from a median of 50 ml of bone marrow from HLA-identical siblings, haploidentical donors, and HLA-mismatched donors. They were infused at a median of 77 days after HSCT. Five patients showed a complete response (CR). The survival of patients with gut aGVHD who received MSCs was significantly better than that of the untreated patients. Prasad *et al.* reported the treatment of 12 pediatric patients with steroid-resistant aGVHD with MSCs.<sup>36</sup> MSCs derived from bone marrow of HLA-mismatched unrelated donors (third-party MSCs, Prochymal) were provided by Orsiris Therapeutics, Inc. MSC therapy was started at a me-

**Table 2.** Treatment of steroid-resistant acute graft-versus-host disease with mesenchymal stromal cells

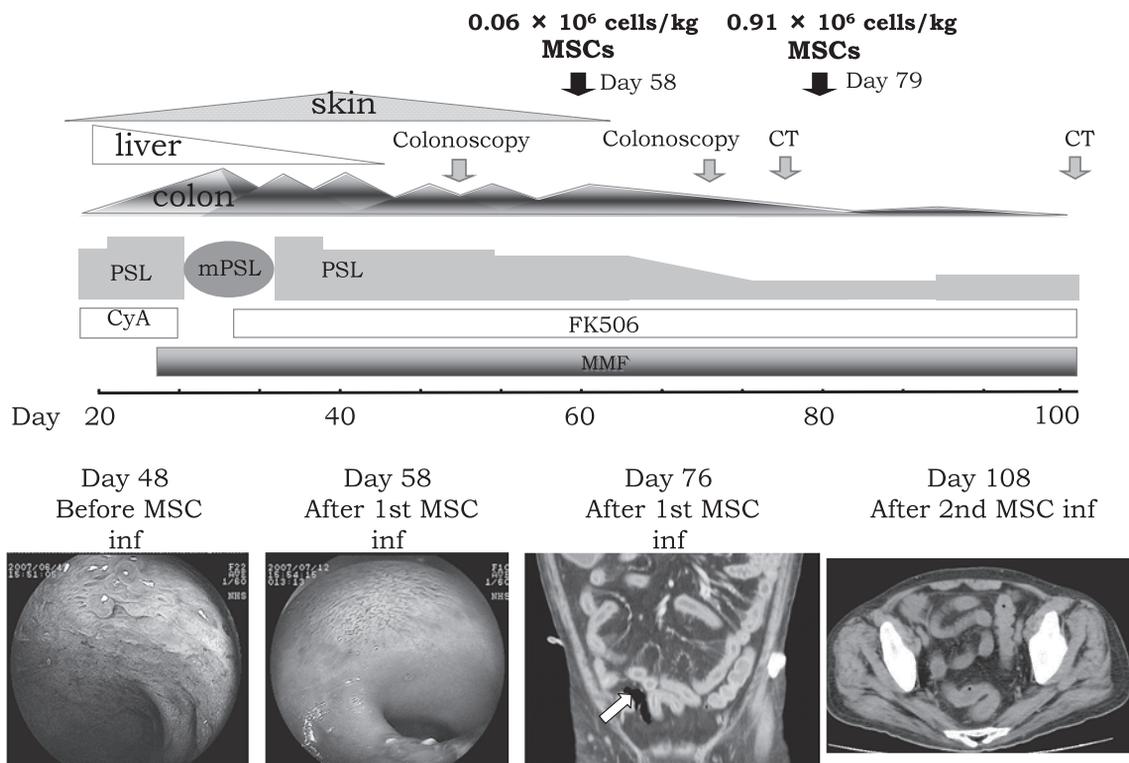
I st author	Year of publication	Ref	No. of pts	Age (Years)	No. of pts with aGVHD III/IV	No. of MSC donors S/Haplo/U	No. of pts for MSC passages Once/ Twice/ More	No. of pts for MSC infusions Once/ Twice/ More	1 st MSC infusion Days after HSCT	No. of infused MSC doses ( $\times 10^6/\text{kg}$ )	No. of pts with response CR/ PR/ Others (%)	Outcome (No.) Alive/Death
Le Blanc	2004	10	1	9	0/0/1	0/1/0	0/1/0	0/1/0	73	1.5	1 (100)/0/0	1/0
Ringden	2006	35	8	56	0/6/2	4/0/4	3/4/4	5/3/3	77	1.3	5 (63)/0/3 (38)	5/3
Ringden <sup>#</sup>	2006	35	16	40	2/10/4	ND	ND	ND	ND	ND	ND	ND
Prasad	2007	36	12	6	0/7/5	0/0/12	0/0/12 (8 times)	0/0/12 (8 times)	119	2 (10 pts), 8 (2 pts)	6 (50)/6 (50)/0	6/6
Fang	2007	37	6	40	0/2/4	0/2/4	ND	5/1/0	71	1	5 (83)/0/1 (17)	4/2
Müller	2008	38	2	9	1/1/0	0/1/1	ND	2/0/0	89	1.7	0/0/2 (100)	1/1
Le Blanc	2008	11	55	22	5/25/25	5/18/69	14/42/36 <sup>#</sup>	27/22/6	ND	1.4	30 (55)/9 (16)/16 (29)	21/34
von Bonin	2009	39	13	58	0/2/11	0/0/13	ND <sup>§</sup>	0/13/0	41 <sup>f</sup>	0.9	1 (8)/1 (8)/11 (85)	4/9
ND	2009	40	260	ND	ND	0/0/260	ND	ND	ND	2	CR (40)	ND
Muroi	2009	41	2	37.5	0/0/2	2/0/0	0/0/2	0/2/0	42	1.0	0/0/2 (100)	0/2
Kebrt <sup>††</sup>	2009	42	31	52	2/17/3	0/0/31	ND	0/31/0	ND	2 (16 pts), 8 (15 pts)	24 (77)/5 (16)/2 (6)	22/9
ND <sup>‡</sup>	2009	43	192	ND	ND	0/0/192	ND	ND	ND	2	ND	ND

Ref. references; Pts, patients; No., number; aGVHD, acute graft-versus-host disease; MSC, mesenchymal stromal cells; S, sibling donors; Haplo, haploidentical donors; U, unrelated donors; HSCT, hematopoietic stem cell transplantation; CR, complete response; PR, partial response; Others; response including minimal response, no response, progression, and no evaluable cases; ND, not done or not shown; <sup>#</sup>, controls; <sup>†</sup>, no. of MSC infusions for MSC passages; <sup>‡</sup>, including after donor lymphocyte infusion and cessation of immunosuppressants; <sup>§</sup>, MSC with or without steroid as a first line therapy for aGVHD

dian of 81 days after HSCT. All patients responded to the therapy with 6 patients having a CR and the rest, a partial response (PR). The application of MSCs derived from adipose tissue to 6 patients with steroid-resistant aGVHD was reported.<sup>37</sup> The median age of the patients was 40 years. The MSCs were obtained from either haploidentical or unrelated donors. The cells were isolated from abdominal adipose tissue of the donors by lipectomy, and cultured with an expansion medium. Five patients showed a CR, four of which were alive and disease-free following infusions of the adipose-derived MSCs. Müller *et al.* reported the response of bone marrow-derived MSCs to various conditions after HSCT.<sup>38</sup> MSCs were isolated with 20 mL of bone marrow and cultured in an expansion medium. One of two pediatric patients with steroid-resistant aGVHD did not develop chronic GVHD (cGVHD). The European Group for Blood and Marrow Transplantation reported a phase II study of bone marrow-derived MSCs for steroid-resistant aGVHD.<sup>11</sup> The pediatric and adult patients numbered 25 and 30, respectively. The median age of all the patients was 22 years. MSC donors included HLA-identical siblings, haploidentical donors, and HLA-mismatched unrelated donors. Mononuclear cells were isolated from a median of 60 mL of bone marrow collected from MSC donors and cultured to obtain MSCs in an expansion medium. The MSCs were passaged once for 14 infusions, two or three times for 47 infusions, and three or four times for 29 infusions. The number of infusions was one for 27 patients, two for 22 patients, and more than two for 6 patients. A median number of  $1.4 \times 10^6$  MSCs/kg was infused. A CR was obtained for 68% of the pediatric patients and 43% of the adult patients. The overall response rate of the patients was 70%. The 2-year survival rate of complete responders was significantly better (53%) than that of partial responders plus non-responders (16%). There was no difference in response rates between patients who received MSCs from third-party donors and those who received MSCs from other sources. von Bonin *et al.* reported the treatment of steroid-resistant aGVHD with MSCs.<sup>39</sup> Thirteen patients with a median age of 58 years were treated with MSCs for steroid-resistant aGVHD. MSCs from unrelated donors' bone marrow were expanded in a medium containing 10% human platelet lysate instead of fetal calf serum (FCS). The median time of the first MSC infusion after HSCT was 41 days. A CR, PR, and mixed response were obtained in one patient, one patient, and five patients, respectively. Osiris conducted a phase III study of Procymal for patients with steroid-resistant aGVHD (protocol 280). This trial was a double-blind, placebo controlled study and patients were randomly allocated treatment with Procymal and a placebo at a proportion of two to one. The total number of patients enrolled was 260. MSCs were administered twice a week for 4 wk at  $2 \times 10^6$  cells/kg per infusion. Recently, Osiris published preliminary results of the phase III study.<sup>40</sup> Although there was no

difference between Procymal and placebo at the primary endpoint, the rate of CR was better in the Procymal group than in the control group (40% and 28%, respectively). Procymal significantly improved response rates to liver and gut aGVHD (29% and 88%, respectively). Notably, the Procymal group had more severe GVHD (28%) than the control group (16%).

We conducted a pilot study of the use of MSCs for steroid-resistant aGVHD after HSCT, which was approved by an institutional review board.<sup>41</sup> The MSC donors were only relatives. Eight patients with steroid-resistant aGVHD were enrolled. About 10 mL of bone marrow was aspirated from each donor. Mononuclear cells were isolated using Ficoll-hypaque density gradient centrifugation and suspended in a human MSC expansion medium containing 10% FCS. Cells were cultured at a density of  $1 \times 10^6$ /mL at 37°C in a 5% CO<sub>2</sub> incubator and non-adherent cells were removed. When adherent cells became confluent, they were detached with trypsin and ethylenediaminetetraacetic acid and passaged. The supernatant of harvested MSCs was checked for bacteria, fungi, endotoxin, hepatitis B antigen, hepatitis C antibody, Epstein-Barr virus DNA, cytomegalovirus DNA, and human herpesvirus 6 DNA. A chromosomal analysis of the MSCs was performed. Of eight patients with steroid-resistant aGVHD, the GVHD in five patients was resolved slowly by steroid or by the addition of a bolus of methylprednisolone and/or mycophenolate mofetil. One patient was excluded due to viral pneumonia. Two patients were administered MSCs, one of whom showed a minimal response. This patient was a 42-year-old male who had acute myeloblastic leukemia (AML) which progressed from myelodysplastic syndrome and did not enter into complete remission (Fig. 2). He received a peripheral blood stem cell transplant from his HLA-identical sister after myeloablative conditioning. The GVHD prophylaxis was short-term methotrexate and cyclosporine treatment. On day 14 after transplantation, a donor-cell engraftment was observed using fluorescent *in situ* hybridization for X and Y chromosomes. On day 18, 1 mg/kg/day of prednisolone was started for grade II aGVHD of skin, liver and gut. On day 22, the aGVHD had progressed despite of prednisolone treatment. Therefore, mycophenolate mofetil was added and the dose of prednisolone was increased. The aGVHD worsened and bloody diarrhea with abdominal cramps occurred. The pathological findings of the colon mucosa were compatible with aGVHD. A bolus of methylprednisolone was given and cyclosporine was changed to tacrolimus. Following these treatments, the skin and liver aGVHD were resolved. However, the gut aGVHD persisted with bloody diarrhea and severe abdominal cramps. Therefore, MSCs were prepared from bone marrow of the same peripheral blood stem cell transplant donor. On day 58,  $0.06 \times 10^6$ /kg of thawed MSCs were infused, however, the abdominal cramps and bloody stools persisted. On day 74, the patient complained of severe abdominal pain. Computed tomography showed free-air in



**Fig. 2.** Clinical course of the patient. MSCs, mesenchymal stromal cells; MSC inf, MSC infusion; CyA, cyclosporine; FK506, tacrolimus; MMF, mycophenolate mofetil; PSL, prednisolone; mPSL, methylprednisolone; CT, computed tomography. *The white arrow indicates free-air due to the intestinal perforation.*

the area surrounding the small intestine due to perforation of the intestine. To resolve the gut aGVHD and repair the intestinal mucosa,  $0.91 \times 10^6/\text{kg}$  of fresh MSCs were infused on day 79. After the second administration of MSCs, the abdominal free-air disappeared and bloody stools decreased. The patient was able to ingest orally. Since the abdominal pain and bloody diarrhea did not completely disappear, infliximab was given on day 153. Although the patient was discharged on day 178, he died of septic shock on day 193.

There are two reports on using MSCs as a first line treatment for aGVHD (Table 2). Kebriaei *et al.* reported treatment of aGVHD with a combination of steroids and third-party MSCs (Procymal).<sup>42</sup> Patients were randomized to either a high-dose MSC group ( $8 \times 10^6/\text{kg}$ ) or a low dose MSC group ( $2 \times 10^6/\text{kg}$ ). The number of patients and the median age in the former group were 15 and 49 years, respectively, while those in the latter group were 16 and 53 years, respectively. There was no difference between the two groups in the overall CR rate and in the CR rate according to the organ system of aGVHD. Osiris conducted a phase III trial of MSCs (Procymal) plus steroid as a first line treatment for aGVHD (protocol 265). One hundred and ninety-two patients were enrolled but the results have not been released.<sup>43</sup>

None of the above reports mentioned above showed im-

mediate or late adverse effects associated with MSC infusions such as infusion reactions, pulmonary embolisms, transmissions of infectious agents, and ectopic mass formation derived from the infused MSCs. Since MSCs are suggested not to cause systemic immunosuppression, it is likely that the graft-versus-leukemia (GVL) reaction is not impaired and the frequency and severity of systemic infections do not increase after MSC therapy. Indeed, none of the above reports indicated a significant increase in relapse or infections after MSC therapy. The effects of MSCs on aGVHD seem not to be associated with MSC origins, i.e., HLA-identical siblings, haploidentical family donors, HLA-matched unrelated donors, and HLA-mismatched (third party) donors. Because it takes time to obtain MSCs by culture, frozen MSCs from third party donors are most suitable for the treatment of aGVHD. MSCs seem to be useful for steroid-resistant aGVHD as a second line therapy, especially for children and for gut aGVHD. In Japan, a phase I/II study of MSCs from third party donors to treat steroid-resistant aGVHD is being conducting.

### ***MSCs for prevention of graft failure, enhancement of engraftment, and prevention of GVHD***

MSCs were cotransplanted with HSCs to prevent graft failure, enhance engraftment, and reduce GVHD (Table 3). A first case was reported by Lee *et al.* in 2002.<sup>44</sup> A 20-year-old woman with high-risk AML was transplanted with peripheral blood CD34<sup>+</sup> cells from her haploidentical father with bone marrow-derived MSCs from the same donor. Engraftment was rapid and no GVHD occurred. Lazarus *et al.* reported the cotransplantation of HSCs from HLA-identical siblings with bone marrow-derived MSCs from HLA-identical siblings.<sup>45</sup> Nineteen patients and 27 patients received bone marrow transplants and peripheral blood stem cell transplants, respectively. The GVHD prophylaxis was short-term methotrexate and cyclosporine treatment. The infused MSC dose was  $1.0 \times 10^6/\text{kg}$  for 18 patients,  $2.5 \times 10^6/\text{kg}$  for 19 patients, and  $5.0 \times 10^6/\text{kg}$  for 5 patients. Neutrophil engraftment and platelet engraftment took 14.0 days and 20.5 days, respectively. aGVHD was observed in 23 patients (50%), of whom 13 (28%) showed grade II to IV aGVHD. Of 21 evaluable patients, 14 and 8 patients had limited and extensive cGVHD, respectively. Relapse or disease progression occurred in 12 patients. Differences between the doses of MSCs in clinical outcomes were not apparent. This study did not show significant rapid engraftment of HSCs or reduction of GVHD. Le Blanc *et al.* reported seven patients with cotransplantation of HSCs with bone marrow-derived MSCs.<sup>46</sup> MSC donors were HLA-identical siblings or haploidentical relatives. The infused MSC dose was  $1 \times 10^6/\text{kg}$ . Three patients had received HSC transplants before the cotransplantation of HSCs and MSCs. Engraftment of the three patients was shown. The cotransplantation of haploidentical HSCs with MSCs was reported by Ball *et al.*<sup>47</sup> The patients were children with the median age of 8 years. They received peripheral blood CD34<sup>+</sup> cells from haploidentical relatives, followed by bone marrow-derived MSCs from the same donors. The mean dose of MSCs was  $1.6 \times 10^6/\text{kg}$ . Engraftment was rapid and graft failure did not occur. aGVHD was shown in 2 patients (14%) for grade I to II, while cGVHD was shown in one patient (7%). These results were not significantly better than historical controls. Ning *et al.* conducted a randomized study comparing HSCT with HSCT plus MSC transplantation.<sup>48</sup> Both HSCT donors and MSC transplantation donors were HLA-identical siblings. The HSCT sources were bone marrow in 9 patients, peripheral blood stem cells in 13 patients, and bone marrow combined with peripheral blood stem cells in 3 patients. Fifteen patients underwent HSCT only, while 10 patients underwent the cotransplantation of HSCs with MSCs. The median infused MSC dose was  $0.33 \times 10^6/\text{kg}$ . Neutrophil engraftment in the HSCT group and the cotransplantation group took 15 and 16 days, respectively. Platelet engraftment in the former and the latter took 27 and 30 days,

respectively. Only grade I or II aGVHD occurred in 11 patients of the former group and 4 patients of the latter group, respectively. cGVHD was shown in 4 of 14 patients in the former and one of 7 patients in the latter, respectively. Infection frequencies did not differ between the two groups. Notably, 3 patients in the former group relapsed (20%), while 6 patients in the latter relapsed (60%). Relapse was not associated with the infused MSC dose. Zang *et al.* examined hematological recovery and GVHD severity in patients receiving HSC transplants plus MSC infusions.<sup>49</sup> Twelve patients received peripheral blood stem cell transplants from HLA-identical siblings, followed by MSC infusions from the same donors. The infused doses of peripheral blood CD34<sup>+</sup> cells and MSCs were  $4.34 \times 10^6/\text{kg}$  and  $1.48 \times 10^6/\text{kg}$ , respectively. The GVHD prophylaxis was short-term methotrexate and cyclosporine treatment. Engraftment was rapid; neutrophil and platelet engraftments took 11 and 13.5 days, respectively. Seven and 2 patients developed grade I and grade III/IV aGVHD, respectively. cGVHD was shown in 4 patients. Relapse occurred in 4 patients (30%) including one with chronic myelogenous leukemia (CML) in an accelerated phase, one with CML in blastic transformation, one with AML in second remission and one with ALL in second remission. Seven patients were alive and 5 patients were dead because of relapse or infection. Gonzzalo-Paganzo *et al.* reported an unique clinical trial of the combined transplantation of cord blood, peripheral blood stem cells from unrelated donors, and bone marrow-derived MSCs from the same peripheral blood stem cell donors.<sup>50</sup> Engraftment and aGVHD severity of the patients were similar to those in control patients.

No adverse effect associated with MSCs was not reported in the above studies. Cotransplantation of HSCs with MSCs seems not to markedly enhance neutrophil and platelet engraftments, as compared with historical controls. However, in cases with a risk of graft failure such as heavily transfused patients with aplastic anemia and patients with a history of graft failure, cotransplantation of HSCs with MSCs may accelerate engraftment of the HSCs. Unfortunately, cotransplantation of HSCs with MSCs does not seem to reduce aGVHD. This may be because MSCs do not effect unstimulated lymphocytes before the onset of aGVHD. Further studies are needed of the efficacy of cotransplanted MSCs for the acceleration of HSC engraftment and aGVHD prevention.

### ***MSCs for cGVHD and tissue repair***

A few patients with cGVHD treated with MSCs were reported with variable responses.<sup>35,38</sup> Very recently, Zhou *et al.* reported the efficacy of bone marrow-derived MSCs for 4 patients with sclerodermatous cGVHD.<sup>51</sup> MSCs were administered by intrabone marrow injection. Following an increase in Th1 lymphocytes and decrease in Th2 lymphocytes, symp-

**Table 3.** Cotransplantation of hemopoietic stem cells with mesenchymal stroma cells to prevent graft failure and/or graft-versus-host disease

1st author	Year of publication	Ref	No. of Pts	Age (Years)	No. of BM-MNCs ( $\times 10^8/\text{kg}$ )	No. of PB-CD 34 <sup>+</sup> ( $\times 10^6/\text{kg}$ )	No. of MSC dose ( $\times 10^6/\text{kg}$ )	Neut engraft (Days)	Plt engraft (Days)	No. of graft failure	No. of pts with aGVHD I/II/III/IV (%)	No. of pts with cGVHD Limited/Extensive	No. of relapse (%)
Lee	2002	44	1	20	ND	5.7	1.5	12	1.5	none	none	none	none
Lazarus	2005	45	46	44.5	3.6	5	1.0 (18 pts), 2.5 (19 pts), 5 (5 pts)	14	20.5	0	3 (7)/13 (28)/5 (11)/2 (4)	14/8	12 (26) <sup>†</sup>
Le Blanc	2007	46	7	12	NS	7.2 <sup>§</sup>	1	12	12 <sup>†</sup>	0	4 (57)/2 (29)/0/0	1/0	0
Ball	2007	47	14	8	ND	21.5	1.6	12	10	0	2 (I+II, 14)/0 (III+IV, 0)	1/0	2
Ball <sup>#</sup>	2007	47	47	7.1	ND	24.3	ND	13	13	7	12 (I+II, 26)/2 (III+IV, 4)	4/2	ND
Ning	2008	48	10	38	5.3 (4 pts)	5.4 (6 pts)	0.33	16	30	0	3 (30)/1 (10)/0/0	1/0	6 (60)
Ning <sup>#</sup>	2008	48	15	37	4.4 (5 pts)	5.1 (10 pts)	ND	15	27	0	3 (20)/8 (53)/0/0	1/1	3 (20)
Zhang	2009	49	12	38.5	ND	4.34	1.48	11	13.5	0	7 (58)/1 (II-III, 8)/1 (8)/0	2/2	4 (30)
Gonzalo-Daganzo	2009	50	9	32	ND	0.12 <sup>§</sup>	1.2	12	44	0	1 (11)/4 (44)/0/0	1/0	1 (11)
Gonzalo-Daganzo <sup>#</sup>	2009	50	46	35	ND	0.10 <sup>§</sup>	ND	10	32	ND	18 (37)/5 (11)/3 (7)/3 (7)	8/3	6 (13)

Ref, references ; Pts, patients ; No., number ; BM-MNCs, bone marrow nucleated cells ; PB-CD 34<sup>+</sup>, peripheral blood CD 34<sup>+</sup> cells ; MSC, mesenchymal stromal cells ; Neut Engraft, days of neutrophil count greater than  $0.5 \times 10^9/\text{L}$  ; Plt engraft, days of platelet count greater than  $20 \times 10^9/\text{L}$  ; aGVHD, acute graft-versus-host-disease ; cGVHD, chronic graft-versus-host disease ; †, including progression ; ND, not done or not shown ; §, including the cases of bone marrow and cord blood cells ; ‡, days of platelet count greater than  $30 \times 10^9/\text{L}$  ; §, cord blood ; #, controls

toms of the patients improved. No adverse effects associated with MSC infusions were noted. It is necessary to clarify whether MSCs are effective against cGVHD and which route of injection is better, a conventional intravenous injection or an intrabone marrow injection.

MSCs are shown to have the ability to repair damaged tissue by homing to damaged sites and differentiating into the cells of that tissue.<sup>10,35</sup> A clinical trial was conducted to repair damaged tissue associated with HSCT or aGVHD using MSCs.<sup>52</sup> Infusions led to a dramatic resolution of hemorrhagic cystitis, gut perforation and pneumothorax after HSCT. Our case, as shown in Fig. 2, showed a resolution of intestinal perforation associated with gut aGVHD on the infusion of MSCs. Although it is not clear which damaged tissues or organs MSCs can repair, MSCs have a promising future to treat damaged tissue following HSCT.

### CONCLUSIONS AND FUTURE DIRECTIONS

MSCs lead to a normalization of the immune system in stimulated mice and humans via inhibition of T cell proliferation, inhibition of inflammatory cytokine production, increase of Treg cells and correction of the Th1/Th2 balance. However, the mechanisms of MSC-mediated T cell suppression are complex and remain unclear. Efforts to clarify the factors or molecules associated with MSC-mediated T cell suppression should be continued, since direct medication to suppress T cell proliferation could be used instead of MSCs. MSCs seem not to suppress the whole immune system but specifically aGVHD without impairment of the GVL effect in leukemia patients. However, there are many unsolved problems in the treatment of GVHD with bone marrow-derived MSCs; the source of MSCs, i.e., the same HSCT donors, haploidentical donors or third party donors, the single dose of MSCs, the total dose of MSCs and the interval of MSC administration. It is unclear whether MSCs preferentially suppress gut aGVHD or aGVHD in pediatric patients. Although there have been no reports on direct MSC-related adverse effects such as infusion reactions, pulmonary embolisms, pathogen transmissions and ectopic tumor formation, careful observations and long-term follow-up for patients receiving MSCs are needed. Finally, both basic researching MSCs and clinical trials using MSCs will lead to bring a better understanding of MSCs in the field of clinical immunology and hematology.

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